植物研究雑誌
J. Jpn. Bot.
83: 295–300 (2008)

Chromosome Numbers and Karyomorphology of Three Species of the Genus *Euphorbia* L. (*Euphorbiaceae*) in the Sikkim Himalaya

Hiroshi IKEDA[a], Takahide KUROSAWA[b] and Hideaki OHBA[a]

[a]Department of Botany, University Museum, University of Tokyo,
Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033 JAPAN;
[b]Faculty of Symbiotic Systems Science, Fukushima University, Fukushima 960-1296 JAPAN

(Received on May 10, 2008)

Somatic chromosome numbers and karyomorphology of three species belonging to the genus *Euphorbia* section *Tulocarpa* and section *Holophyllum* collected from the Sikkim Himalaya are reported. *Euphorbia sikkimensis* Boiss. (sect. *Tulocarpa*) was found to be 2n = 26 (diploid), *E. griffithii* Hook. f. (sect. *Tulocarpa*) 2n = 52 (tetraploid), and *E. luteoviridis* D. G. Long (sect. *Holophyllum*) 2n = 20. Chromosome numbers of *E. griffithii* and *E. luteoviridis* were examined for the first time. The chromosome number of *E. sikkimensis* was different from those in previous reports. Chromosomes of *E. sikkimensis* and *E. griffithii* were different in ploidy level. Polyploidy may play an important role in species diversification in *Euphorbia* sect. *Tulocarpa* in the Sikkim Himalaya.

Key words: Chromosome number, cytology, *Euphorbia*, Himalaya, karyomorphology.

In the Himalayas, relatively few *Euphorbia* L. species are found in the alpine vegetation (Ohba 1988), although it is a cosmopolitan genus comprising about 2000 species and one of the largest genera of angiosperms (Govaerts et al. 2000, Radcliffe-Smith 2001). Kurosawa (2002) enumerated eight alpine species of *Euphorbia* in the Nepal Himalaya and Sikkim Himalaya, and all of them are endemic to the Himalayas. They belong to two sections in subgenus *Esula*: *E. sikkimensis* Boiss., *E. griffithii* Hook. f., *E. pseudosikkimensis* (Hurus. & Ya. Tanaka) Radcl.-Sm., and *E. cashmeriana* Royle to section *Tulocarpa* (Raf.) Prokh., and *E. wallichii* Hook. f., *E. luteoviridis* D. G. Long, *E. himalayensis* (Klotzsch) Boiss., and *E. stracheyi* Boiss. to section *Holophyllum* Prokh. They are relatively common in alpine and subalpine scrubs and meadows (Lanchaster 1995) as a result of their successful adaptation to growing in more or less grazed habitat at high altitude.

Recent investigations of chromosome numbers and karyotypes have revealed various cytological features in the Himalayan taxa. Polyploid series have been found in *Potentilla* sect. *Leptostylae* (*Rosaceae*) (Ikeda and Ohba 1999, Ikeda 2002) and in some groups of *Saxifraga* (*Saxifragaceae*) (Wakabayashi and Ohba 1988, Wakabayashi 1997). Wakabayashi (2002) noted that polyploidy and aneuploidy were found in *Rhodiola bupleuroides* (Wall. ex Hook. f. & Thomson) S. H. Fu (*Crassulaceae*) and morphologically different forms were correlated with ploidy levels. Those examples are suggested that polyploidy/aneuploidy may play an important role(s) in speciation in some groups in the Himalayas. On the other hand, in *Impatiens* (*Balsaminaceae*), a morpho-

Table 1. Locality, voucher specimen and somatic chromosome number of three species of *Euphorbia* in the Sikkim Himalaya studied in this study

Taxon	Locality	Voucher specimen	2n
E. sikkimensis	Chana–Thollung Monastery, alt. 2400 m	Miyamoto & al. 20390160	26
	Chana–Thollung Monastery, alt. 2400 m	Miyamoto & al. 20390161	26
E. griffithii	Thollung Monastery–Tamrong, alt. 2820 m	Miyamoto & al. 20390171	52
E. luteoviridis	Tamrong–Dikillnang, alt. 2890 m	Miyamoto & al. 20390176	20

logically diverse genus in the Himalayas, all the examined species have been reported as diploids (Akiyama et al. 1992). For clarifying cytological features in Himalayan plants, it is necessary to examine various taxa.

Although the chromosome numbers of *Euphorbia* subgenus *Esula* have been reported by many authors, cytological records for the Himalayan *Euphorbia* are still limited. Only one species for each section has been reported: *E. sikkimensis* (sect. *Tulocarpa*) as n = 12 and 2n = 24 (Sharma and Saker 1967–1968, Sharma 1970, Roy et al. 1988), and *E. wallichii* (sect. *Holophyllum*) as n = 10 and 2n = 20 (Sapru and Kaul 1971, Mehra and Choda 1978). This paper reports chromosome numbers and karyomorphology of the three species, *E. sikkimensis*, *E. griffithii* (sect. *Tulocarpa*), and *E. luteoviridis* (sect. *Holophyllum*), to add to our knowledge on cytological features of the two sections of *Euphorbia* in the Himalayas. Chromosome numbers of *E. sikkimensis* have already been reported but it is a poorly-known and sometimes misunderstood species (Long 1987). We examined materials from near the type locality in temperate Sikkim. Long (1987) suggested *E. griffithii* as a subspecies of *E. sikkimensis*. Cytological features of the materials of the two species from the same area may reveal genetical isolation present among them. Cytological studies of *Euphorbia* sect. *Holophyllum* have been done mainly on materials collected in Kashmir in the west Himalayas (Sapru and Kaul 1971, Mehra and Choda 1978). This time we choose *E. luteoviridis*, an east Himalayan species of the section, for this study, which has never been reported the chromosome number.

Materials and Methods

Plants were collected from their native habitat in the Sikkim Himalaya in 2003 (see Noshiro 2004). Localities and voucher specimens are listed in Table 1. Voucher specimens used in this study will be deposited in the Herbarium at the University of Tokyo (TI).

Chromosome counts were made on somatic metaphase cells using the squash technique. Four populations of three species were analyzed. Root tips were fixed with Newcomer's fluid (see Sharma and Sharma 1980) in the field after pre-treatment in 2 mM 8-hydroxyquinoline solution for about 3–4 hr. The fixed roots were cleaned with an alcohol series and distilled water, then hydrolyzed with 1 N HCl at 60ºC for 10 minutes and stained with leuco-basic fuchsin for 1 hour. In order to macerate cell walls, the samples were soaked in a solution of 2% pectinase and 2% cellulase (1:1) for 2–3 hr. at 37ºC and stained with 2% lacto-propionic orcein for several minutes and squashed.

Results and Discussion

The chromosome numbers of three species of *Euphorbia* subg. *Esula* are shown in Table 1. Chromosomes observed in a somatic cell of each species are shown in Fig. 1.

1. *Euphorbia sikkimensis* Boiss.

[Fig. 1, a, a']

The somatic chromosome number in this species was 2n = 26. This number is considered diploid, with basic chromosome number x = 13. Choromosomes are 2.4–7.4 μm long. Karyomorphologically, the complement is bimodal, comprises one pair of relatively large chromosomes (7.2–7.4 μm long) and 12 pairs of relatively small chromosomes (2.4–5.7 μm long). No satellite chromosome was observed.

Although *Euphorbia* sect. *Tulocarpa* shows diversity in somatic chromosome numbers 2n = 14, 28, 56 prevail and 2n = 26 is relatively rare (Kurosawa unpublished).

Sharma and Sarker (1967–1968) and Sharma (1970) reported somatic chromosome number of *E. sikkimensis* as 2n = 24, and Roy et al. (1988) reported gametophytic chromosome number of this species cultivated in Lloyd Botanical Garden, Darjeeling, as n = 12. These numbers are different from the result of this study. There are several possibilities for this incongruity: 1) misidentification for the species, 2) miscounting for chromosomes, and 3) infraspecific variation in chromosome number in *E. sikkimensis*. For confirmation of the chromosome number of *E. sikkimensis*, it is necessary to make a cytological investigation covering whole range of the species.

2. *Euphorbia griffithii* Hook. f.

[Fig. 1, b, b']

The somatic chromosome number was 2n = 52. This number is considered tetraploid, with basic chromosome number x = 13. Chromosomes are 2.2–8.9 μm long. Karyomorphologically, the complement is bimodal, comprises two pairs of relatively large chromosomes (7.3–8.9 μm long) and 24 pairs of relatively small chromosomes (2.2–5.6 μm long). No satellite chromosome was observed. The chromosome number of this species is reported for the first time.

Euphorbia griffithii is similar to *E. sikkimensis* in gross morphology. Long (1987) suggested that these two species may only be distinguished at subspecific rank. *Euphorbia griffithii* is, however, distinguished from *E. sikkimensis* in having orange-red flower heads and shorter stem as pointed by Hooker (1886–1888) and Turner (1995). From the result of this study, it is clarified that *E. griffithii* and *E. sikkimensis* are different in ploidy level: diploid for *E. sikkimensis* and tetraploid for *E. griffithii*. They are genetically isolated from each other and can be distinguished by some morphological characters, therefore they should be treated as two independent species. However, karyomorphologically they possess similar karyotypes. They both have bimodal complements in chromosome lengths, with one pair of relatively large chromosomes in diploid species, *E. sikkimensis*, and two pairs in tetraploid species, *E. griffithii*. It is thought that *E. griffithii* is an autotetraploid species that arose from diploid ancestral species which possessed a bimodal complement in chromosome length as *E. sikkimensis* has. It is possible that polyploidy plays an important role in speciation of sect. *Tulocarpa* in the Himalayas as in *Potentilla* sect. *Leptostylae* (Ikeda and Ohba 1999, Ikeda 2002) and *Saxifraga* sect. *Ciliatae* (Wakabayashi 1997).

3. *Euphorbia luteoviridis* D. G. Long

[Fig. 1, c, c']

The somatic chromosome number was 2n = 20. This number is considered diploid, with basic chromosome number x = 10. Chromosomes are 4.7–9.4 μm long. Karyomorphologically, the complement is monomodal, gradually decreasing in size. Satellites were observed in two pairs of chromosomes: in the longest and the next longest pairs. The chromosome number of this species is reported for the first time.

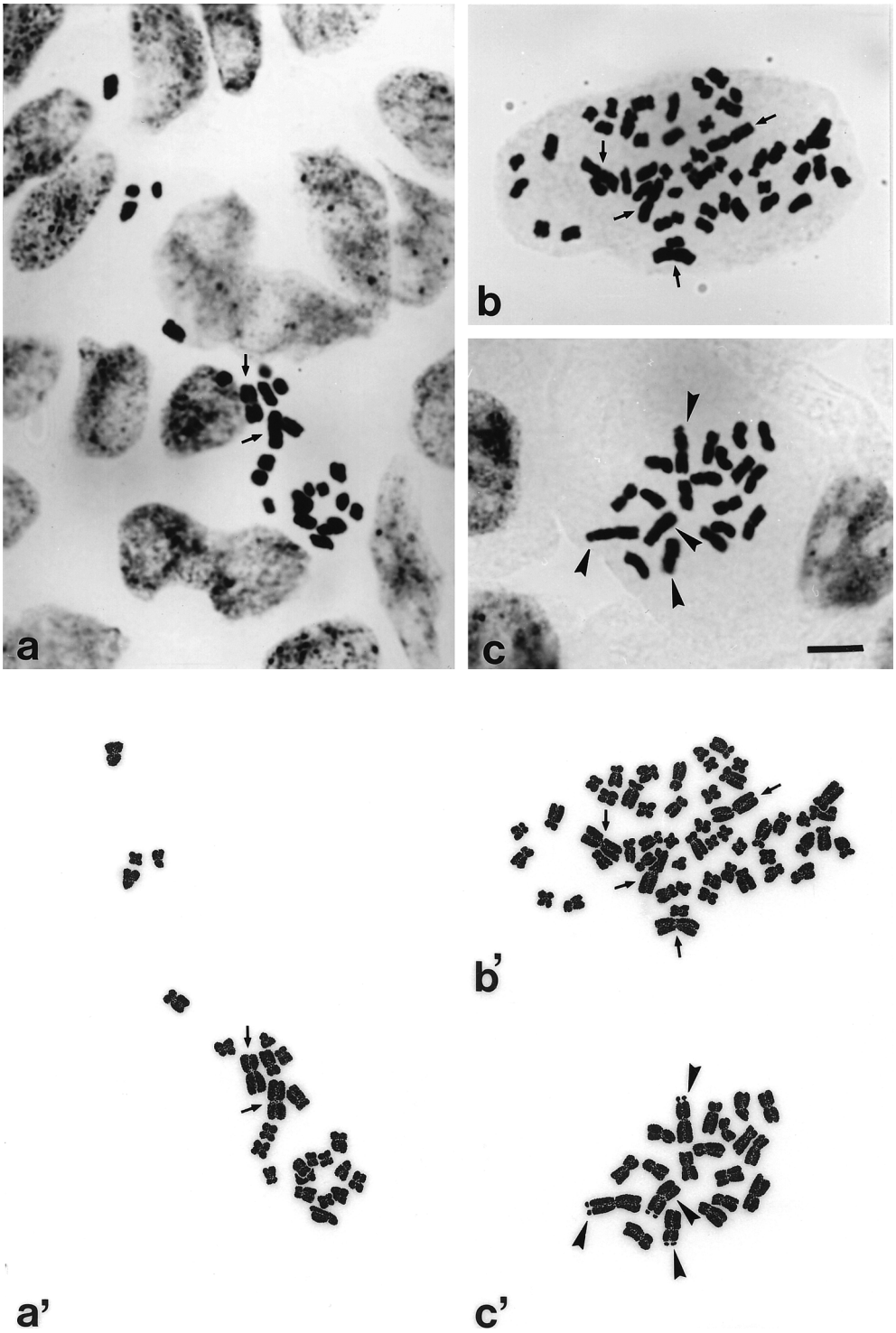

Fig. 1. Somatic metaphase chromosomes of three species of *Euphorbia* in the Sikkim Himalaya. a, a'. *E. sikkimensis* (2n = 26). b, b'. *E. griffithii* (2n = 52). c, c'. *E. luteoviridis* (2n = 20). Arrow indicates large chromosome. Arrowhead indicates satellite chromosome. Bar indicates 5 μm.

Euphorbia luteoviridis shares the same chromosome number with its closely related Himalayan species, *E. wallichii*. The basic number x = 10 was reported for other members of *Euphorbia* sect. *Holophyllum*: 2n = 20 for *E. ebracteolata* (Nishikawa 1990, Chung et al. 2003), 2n = 20 for *E. fischeriana* (Chung et al. 2003, as *E. pallasii*), with the exception of 2n = 18 for *E. rupestris* (Sopova et al. 1983). In contrast with *Euphorbia* sect. *Tulocarpa*, the section has no reported polyploid species. Further studies are, however, needed for clarifying the cytological diversification in the section, since the chromosome numbers have been reported only for five of about 30 species included in the section.

We are grateful to the Department of Forests, Environment and Wildlife, Government of Sikkim, India for the help in carrying out the field survey. This study was partly supported by Grants-in-Aid for Scientific Research (A) no. 14255005 and 18255004 to H. O. from the Japan Society for the Promotion of Science.

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池田 博[a]，黒沢高秀[b]，大場秀章[a]：**シッキムヒマラヤに産するトウダイグサ属（トウダイグサ科）3種の染色体数と核型**

シッキムヒマラヤに産するトウダイグサ属タカトウダイ節（*Euphorbia* sect. *Tulocarpa*）およびマルミノウルシ節（*Euphorbia* sect. *Holophyllum*）3種の染色体数を報告した．染色体数はタカトウダイ節の *E. sikkimensis* が 2n = 26，*E. griffithii* が 2n = 52，マルミノウルシ節の *E. luteoviridis* が 2n = 20 であった．*E. griffithii* と *E. luteoviridis* については，今回が初めての報告である．*E. sikkimensis* の染色体数は，これまでの報告と異なっていた．近縁な *E. sikkimensis* と *E. griffithii* の間で染色体数の倍数化が見られた．今回の結果は，ヒマラヤにおいてトウダイグサ属タカトウダイ節の植物が染色体の倍数化を伴う多様化を起こしている可能性を示している．

（[a]東京大学総合研究博物館植物部門，
[b]福島大学共生システム理工学類）